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Selective Removal of Cholesterol Esters in an Arteriosclerotic Region of Blood Vessels With a Free-Electron Laser

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Background and Objective: **In advanced atherom atherosclerosis, a large amount of lipids, particularly cholesterol esters, accumulates on the arterial wall. The selective removal of cholesterol esters accumulated in the intracellular or extracellular spaces has clinical significance. In the present work, the authors investigated the removal of cholesterol esters by using a free-electron laser (FEL) in an arteriosclerotic region.**
Study Design/Materials and Methods: **Thin films of cholesteryl oleate and albumin and the cross section of a rabbit artery were placed on an inverted microscope stage, and the changes caused by the FEL irradiation of 5.75 μm and 6.1 μm, with 1.5–3 mW on average, were monitored continuously by using a CCD camera in real time.**
Results and Conclusion: **FEL irradiation at a wavelength of 5.75 μm, which is a stretching vibrational mode of the ester, was able to ablate cholesterol esters without affecting albumin. It can also remove cholesterol esters from rabbit arteriosclerotic arterial walls. Lasers Surg. Med. 23:233–237, 1998.**

Key words: ablation; arteriosclerosis; infrared laser; FTIR

INTRODUCTION

Although laser techniques have been attempted for treatment of patients with myocardial infarction, selective ablation of a targeted material from an arteriosclerotic region has been difficult. The removal of cholesterol esters from the arteriosclerotic region has been particularly difficult, because cholesterol bound to fatty acids enters the arterial tissues in a complicated manner. We could not tune the laser beam to a desired wavelength, because the wavelength of most lasers is determined by the type of laser media. The free-electron laser (FEL) is unique, because this laser beam is broadly tunable [1]. At the Free-Electron Laser Research Institute, we have developed an FEL system in which the wavelength may be adjusted to any desired value, ranging from 350 nm to 40 μm [2,3]. We report here the results of experiments in which the FEL was tuned to 5.75 μm, a wavelength that corresponds to the stretch mode of the ester bonds. Cholesterol ester, albumin, and isolated rabbit arterial wall specimens were irradiated. We found that cholesterol ester was ablated selectively without damaging albumin or the normal arterial wall.

MATERIALS AND METHODS

Cholesterol Ester

Cholesteryl oleate, 0.02 g (C-9253, $C_{45}H_{78}O_2$, FS = 651.1; Sigma, St. Louis, MO), was dissolved in 5 ml carbon tetrachloride. An aliquot (10 ml) was dropped on the surface of a BrF2 crystal plate (12 mm diameter, 1 mm thickness). After evapo-

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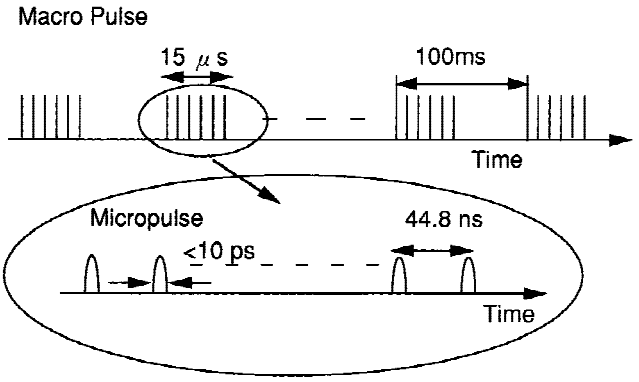

Fig. 1. The structure of free-electron laser (FEL) beam profile. The FEL has two pulse structures, macropulses and micropulses.

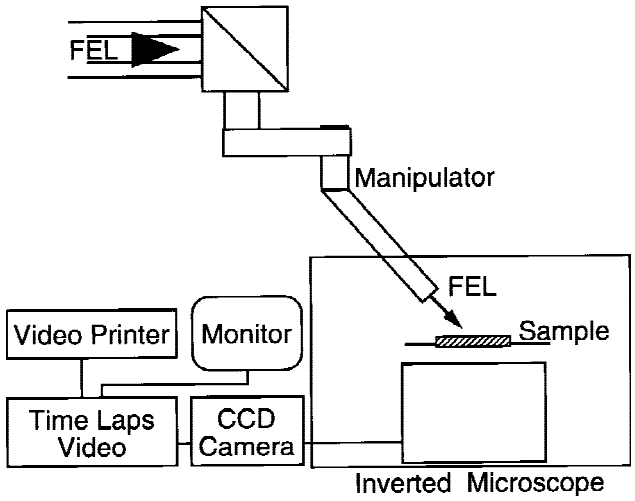

Fig. 2. Experimental set-up for FEL irradiation on samples. Infrared FEL was guided through a pipeline and through a mirror in a mirror chamber close to the ceiling in the laboratory. The guided FEL beam was reduced in size to 0.5 mm in diameter by using ZnSe lenses and concave mirrors. Samples on the stage of an inverted microscope were irradiated by the FEL through a multijointed manipulator. The image from the microscope was caputured by the time-lapse video system using a CCD camera.

ration of the carbon tetrachloride, a thin film of cholesteryl oleate was formed.

Albumin

Human albumin, 0.02 g (A-1887; Sigma; essentially fatty acid free), was dissolved in 0.5 ml ethanol. An aliquot (10 ml) was dropped on the surface of a BrF2 crystal plate (12 mm diameter, 1 mm thickness). After evaporation of the ethanol, a thin albumin film was formed.

Rabbit Arterial Cross Section

New Zealand white rabbits (29 weeks old) were fed with a cholesterol-rich diet (0.5% cholesterol) for 4 weeks. After confirmation that the serum cholesterol level was over 1,200 mg/dl, the carotid artery (3 mm diameter with a wall thickness of 0.5 mm) was obtained. The cross section of the artery was sliced to a thickness of 10 mm by using a cryostat. The sliced specimen was placed immediately on top of the BrF2 crystal plate and dried at room temperature [4].

FEL

The FEL has a complex pulse structure. The structure consists of a train of macropulses, and each macropulse contains a train of 300–400 ultrashort micropulses, as shown in Figure 1. In the present experiment, a width of the macropulse was about 15 μs, and the repetition rate was 10 Hz. Separation between micropulses was 45 ns. The width of the micropulse can be estimated to be shorter than the bunch length of the electron beam, which has been measured as 10 ps in the accelerator operation condition for the mid infrared range. The average power used in these experiments was 1.5 mW with a power density of 1 W/cm^2. The FEL beam was directed to an optical microscope through a flexible, multijoint, cylindrical tube. The beam on the object revealed an oval shape with a long axis of 500 μm and a short axis of 300 μm. A specimen was placed on an inverted microscope stage, and the changes caused by FEL irradiation were monitored continuously by a CCD camera in real time. Images were taken every second by a time-lapse video recorder and were stored on an optical magnetic disk (see Fig. 2).

FTIR

Infrared absorption of the object before and after FEL radiation was determined by microscopic transmission FTIR (model FT-520; Hoshiba, Japan).

RESULTS

Figure 3 shows the effect of FEL radiation on the thin films of cholesterol ester and albumin. In Figure 3a, no radiation damage was noticed when the cholesterol ester was irradiated by an FEL tuned to 6.1 μm, which was not absorbed by the ester bond. However, if the same object was irradiated by an FEL tuned to 5.75 μm, which was absorbed by the ester bond, then radiation dam-

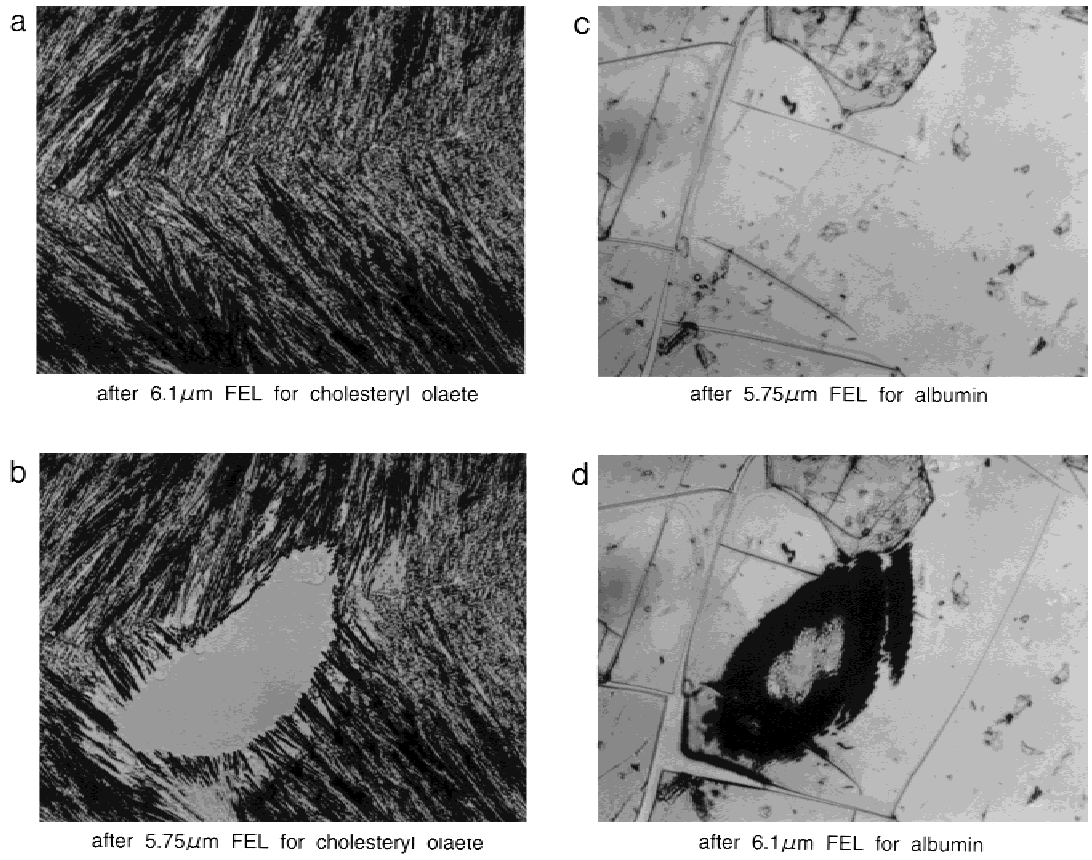

Fig. 3. **a–d:** The effect of FEL irradiation on thin films of cholesteryl oleate and albumin. Samples were exposed to the FEL for 5 seconds each, which became 5 J/cm^2 in fluence.

age was seen instantly (Fig. 3b). Figure 3c shows the results when the thin albumin film was irradiated with an FEL tuned to 5.75 μm, which was not absorbed by the protein. There was no radiation damage. On the contrary, when the same object was irradiated by an FEL tuned to 6.1 μm, which was absorbed by the amide bond of albumin, radiation damage was seen instantly, causing a cavity in the middle of the film due to ablation after 10 seconds of irradiation (Fig. 3d).

Figure 4 shows the effect of the 5.75-μm FEL radiation on the arteriosclerotic area of the rabbit carotid artery followed by irradiation with the 6.1-μm FEL. Irradiation of the 5.75-μm FEL for 4 minutes did not cause any damage to the elastic fiber and its vicinity. However, the 6.1-μm FEL caused carbonization of the fiber after 1 second of irradiation, whereas 16 seconds of irradiation caused carbonization of the arteriosclerotic region where cholesterol esters had accumulated. Figure 5 shows the histology of Figure 4 with further magnification. The top photomicrograph in Figure 5 shows that the irradiation by the 5.75-μm FEL caused no changes in the normal arterial wall, where there is no accumulation of cholesterol ester. However, such irradiation on the arterial wall in which cholesterol ester crystals were present gradually removed the cholesterol esters (Fig. 5, middle). Further irradiation gradually ablated the accumulated cholesterol esters (Fig. 5, bottom). Figure 6 shows the infrared absorption spectra of the thin film of cholesterol ester before and after irradiation by an FEL tuned to 5.75 μm. The irradiation reduced the absorbance at 5.75 μm from 1.75 to 0.4, indicating the degradation of cholesterol ester.

DISCUSSION

In the infrared region, there are peaks in the absorption spectrum of the arterial tissue that correspond to the stretching vibrations of bonds between atoms or molecules. The amide-I bond in the tissue corresponds to a specific absorption peak at 6.1 μm, whereas the amide-II bond corresponds to a peak at 6.45 μm [5], and the ester bond responds to a peak at 5.75 μm [6]. In ad-

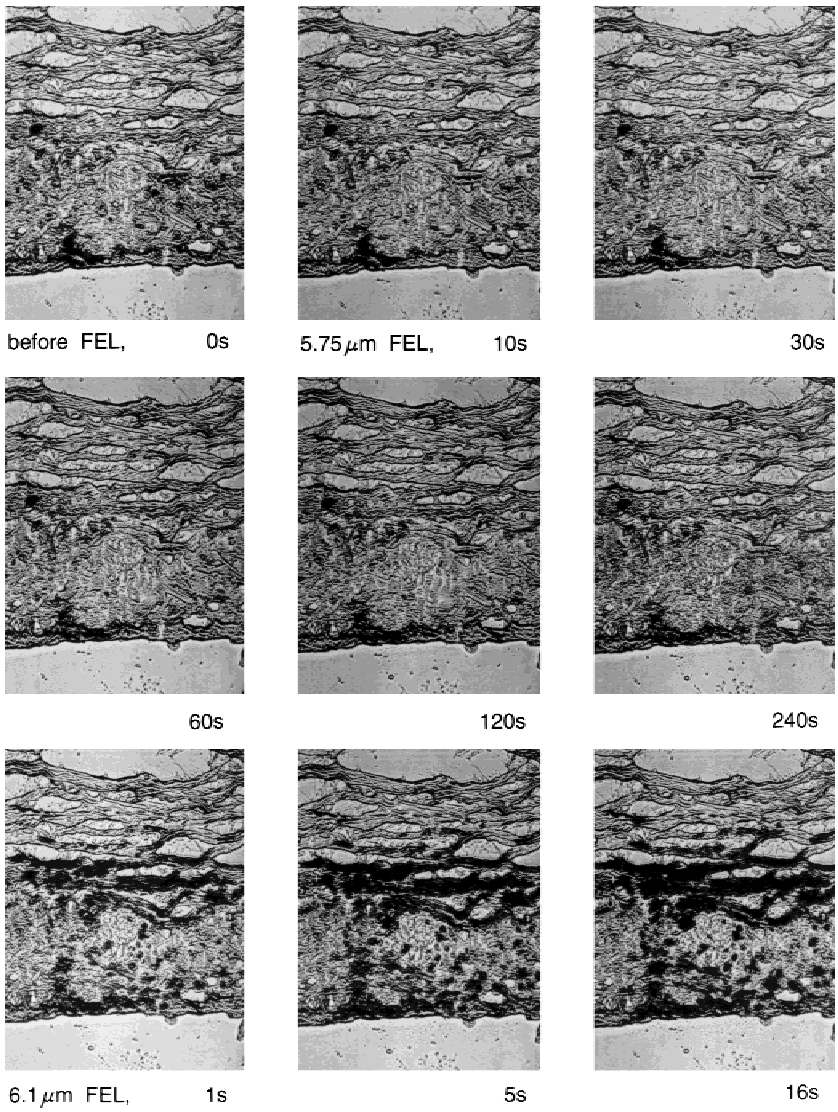

Fig. 4. The effect of FEL irradiation on rabbit artery. **Top:** From left to right, three photomicrographs show a control (no FEL) and samples after FEL irradiation for 10 seconds and for 30 seconds, respectively. **Middle:** From left to right, three photomicrographs show samples after 5.75 μm FEL irradiation for 60, 120, and 240 seconds, respectively. **Bottom:** From left to right, three photomicrographs show the irradiation effect of 6.1 μm FEL for the sample. The sample was exposed previously to 5.75 μm FEL irradiation for 240 seconds.

vanced atherom atherosclerosis, a large amount of lipids, particularly cholesterol esters, accumulates on the arterial wall. In more advanced cases, the lipids also accumulate in the interstitial spaces [7]. The selective removal of cholesterol esters accumulated in the intracellular or extracellular spaces has clinical significance. Our findings that FEL irradiation at a wavelength of 5.75 μm can ablate cholesterol esters without affecting albumin and that it also can remove cholesterol esters from rabbit arteriosclerotic arterial walls lead to the possibility that the laser, which has been used mainly to cut, can also be used for selective ablation of cholesterol esters in the arterial wall.

With regard to the delivery system, this FEL beam in the infrared region is guided by a flexible, multijoint, cylindrical tube. However, the tube is difficult to deliver to small targets in a narrow space, such as an artery, because of the required flexibility of the optics. For this purpose, we propose using chalcogenide glass fibers, which have been developed for thermal measurements and power transmission for infrared lasers [8]. In the

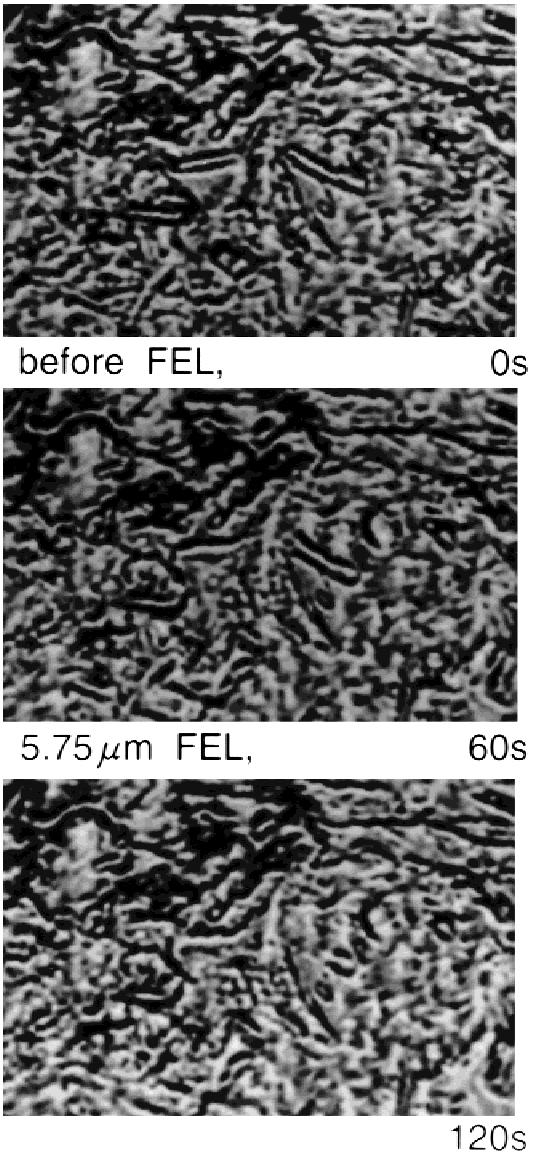

Fig. 5. Cholesteryl oleate, as seen in the middle of the picture, was removed gradually after 5.75 μm FEL.

near future, infrared glass fibers can be used as a delivery tool for angioplasty.

CONCLUSION

We have reported that irradiation with an FEL (wavelength 5.75 μm) can be used to ablate cholesterol esters selectively from the arterial wall.

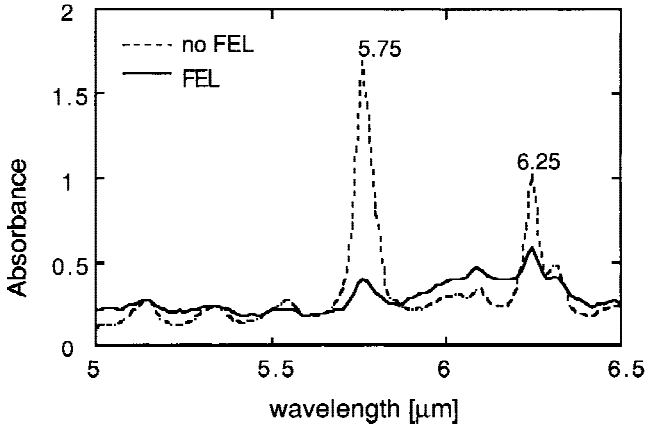

Fig. 6. The infrared adsorption spectra of the thin film of cholesterol ester before and after irradiation with an FEL tuned to 5.75 μm.

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